URINARY STEROIDS IN POST-NATAL ADRENAL HYPERPLASIA WITH VIRILISM

By

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ABSTRACTS

The urinary steroids have been analyzed in a 12-year-old girl with adrenal hyperplasia in whom virilization started at age 6. Androsterone and etiocholanolone levels were elevated and increased normally in response to adrenocorticotrophin (ACTH). The excretion of 11β-hydroxy-androsterone was high and of 11β-hydroxy and 11-ketoetiocholanolone low. She excreted 16 mg of pregnanetriol daily before ACTH, and 48 mg with ACTH. The excretion of tetrahydrocortisol and tetrahydrocortisone was normal and increased with ACTH.

This steroid pattern was compared with that of 3 patients with adrenal hyperplasia and Cushing's syndrome. These patients excreted only slightly increased amounts of pregnanetriol and large amounts of all of the 11-oxyketosteroids.

The occurrence of post-natal virilizing adrenal hyperplasia has been reviewed and the hypothesis offered that there is a congenital partial block of 21-hydroxylation that becomes clinically apparent only some years later.

Although the most common cause of virilization in the female is congenital adrenal hyperplasia, post-natal virilization is rarely due to this process. A recent review (Wilkins 1957) cited only one such case. However, reports by Decourt et al. (1957), Prunty et al. (1958) and Greenblatt (1958) suggested that the biochemical findings characteristic of congenital adrenal virilism may also occur in post-natal virilism. More extensive analysis of these cases confirmed the chemical findings (Jayle et al. 1960; Brooks et al. 1960). The limited information about steroid excretion in such cases and the rarity of post-natal virilizing adrenal hyperplasia, prompted the presentation of our studies.
It is appropriate to compare this patient's steroid excretion and the response to adrenocorticotrophin (ACTH) not only with the normal but also with that of patients with Cushing's syndrome due to adrenal hyperplasia. For this purpose, we have performed similar studies in 3 patients with adrenal hyperplasia. One of these women had Cushing's syndrome accompanied by marked virilization and the other 2 presented the classical form of Cushing's syndrome.

MATERIAL AND METHODS

Twenty-four hour urines were collected in the hospital without preservative and stored at -4°C until processed. In case 1, the urine was frozen and shipped to this laboratory by Air Express. A 24-hour aliquot from each pool was analyzed.

The following abbreviations and trivial names have been used:

- Dehydroepiandrosterone, DHA - 3β-hydroxy-androst-5-en-17-one
- Androsterone, A - 3α-hydroxy-5α-androstan-17-one
- Etiocolcholone, E - 3α-hydroxy-5β-androstan-17-one
- 11β-hydroxyandrosterone, 11-OHA - 3α,11β-dihydroxy-5α-androstan-17-one
- 11β-hydroxyetiocholanone, 11-OHE - 3α,11β-dihydroxy-5β-androstan-17-one
- 11-ketotiocholanolone, 11-KE - 3α-hydroxy-5β-androstan-11,17-dione
- Tetrahydro F - 3α,11β,17α,21-tetrahydroxy-5β-pregnane-20-one
- Tetrahydro E - 3α,17α,21-trihydroxy-5β-pregnane-11,20-dione
- Tetrahydro S - 3α,17α,21-trihydroxy-5β-pregnane-11,20-one
- Pregnanetriol - 5β-pregnane-3α,17α,21α-triol.

Total urinary 17-KS were measured by a modification of the method of Werbin & Ong (1954) and urinary corticoids as Silber-Porter chromogens (S-PC) by a modification of their method (Silber & Porter 1954). Normal values for women in this laboratory are: 17-KS, 5-16 mg/day; S-PC, 3-9 mg/day.

Methods of hydrolysis and extraction have been described (Lipsett & Riter 1960). A preliminary separation of steroid groups was performed on a silicate partition column (Wilson et al. 1958). The 11-deoxy-17-ketosteroids (11-deoxy KS) and the 11-oxy-17-ketosteroids (11-oxy KS) were then separated by paper chromatography (Lipsett & Riter 1960). After elution the ketosteroids were determined by a micro-Zimmermann reaction and the values corrected to milligrams of dehydroepiandrosterone (Wilson 1954). Pregnanetriol was separated by paper chromatography (Lipsett & Riter 1960) after preliminary treatment of the appropriate fraction from the column with Girard’s T reagent (Girard & Sandulesco 1936) and measured as the sulfuric acid chromogen. As a further check on identity, the pregnanetriol in Case 1 before and after ACTH and after ACTH in Cases 2, 3, and 4 was oxidized with sodium bismuthate (Bush & Willoughby 1957) and subsequently shown to have the same running rate as 3α-hydroxy-5β-androstan-17-one.

Tetrahydro F and tetrahydro E were separated in the B3 system (Bush 1952) and tetrahydro S in the E4 system of Eberlein & Bos:igovanni (1955). The steroids and appropriate paper blanks were eluted and determined with blue tetrazolium (Nowaczynski et al. 1955). All results have been expressed as milligrams per 24-hours.

The dose of ACTH given to each patient is stated in the following case histories. The control group was composed of 3 women between the ages of 25 and 35 given 40 IU of ACTH intravenously over 10 hours on 2 successive days.

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Patients: Case 1 was a 12-year-old girl, one of non-identical twins who appeared normal at birth and throughout infancy. About age 6, there was a growth spurt and the development of severe acne, pubic hair, clitoral hypertrophy and a few bluish striae over the buttocks. Urinary 17-KS excretion was 15 mg per day. Laparotomy in Germany at the age of 9 had disclosed normal pre-pubertal ovaries and grossly normal adrenals.

At the time of this study, her chronological age was 12, height age 18, and bone age advanced. Physical examination showed the above features as well as a deep voice and increased axillary and pubic hair. Menarche had not occurred. Urinary 17-KS were 16–18 mg per day, S-PC 3.5–5 mg per day, and pregnanetriol 16 mg per day. After the administration of 37.5 mg of cortisone acetate daily, pregnanetriol excretion was less than 1 mg per day. She received 20 IU of ACTH intravenously over 8 hours daily for 2 days.

Case 2 was a 34-year-old woman with a 1 year history of weight gain, hirsutism, loss of scalp hair, irregular menses, and personality change. Physical examination disclosed a slightly plethoric, balding, hirsute woman. Her muscle strength was good. There was a suggestion of truncal obesity, slight facial rounding and clitoral hypertrophy. The predominant clinical picture was that of virilization accompanied by some features of Cushing's syndrome. Glucose tolerance was decreased. The daily excretion of total 17-KS varied between 28 and 46 mg per day, and of S-PC between 13–17 mg per day. There was a greater than 50 per cent suppression of steroid excretion by Δ1-9α-fluorohydrocortisone. Surgery revealed bilateral adrenal hyperplasia. She received 1 course of 40 IU of ACTH intravenously over 10 hours.

Patients 3 and 4 were 2 women with classical Cushing's syndrome due to bilateral adrenal hyperplasia. Both received 40 IU of ACTH intravenously daily for 3 days.

RESULTS

In Fig. 1, the excretion of the 11-deoxy-17-KS has been plotted for each patient during the control period and during the last day of ACTH. The excretion of dehydroepiandrosterone did not differ from normal in any of the patients either during the control period or after ACTH. The excretion of androsterone and etiocholanolone was above the mean adult range in Case 1 and in the same range as that of Cases 2 and 3. ACTH increased the excretion of these metabolites to the same extent in the control groups and the experimental groups.

The pattern of excretion of the 11-oxy-17-KS differed markedly between Case 1 and Cases 2, 3 and 4 (Fig. 2). In case 1, the excretion of 11-OE and 11-OHE was somewhat less than normal; Cases 2, 3 and 4 excreted increased amounts of these metabolites. Following the administration of ACTH to the subjects with Cushing’s syndrome, there was a greatly augmented excretion of 11-OE and 11-OHE when compared to the response of the normal subjects. The excretion of 11β-hydroxyandrosterone was elevated in all the subjects. The data suggest that ACTH resulted in a greater increase of 11-OHA in Cases 2, 3 and 4 than in Case 1, but too few studies have been performed to demonstrate this with certainty.
Fig. 1.
Excretion of 11-deoxy KS and the response to ACTH.

Fig. 2.
Excretion of 11-oxy KS and the response to ACTH.
Excretion of pregnanetriol, tetrahydro S, tetrahydro E, and tetrahydro F and the response to ACTH.

In Fig. 3, the excretion of pregnanetriol, tetrahydro S, tetrahydro E, and tetrahydro F has been diagrammed. The striking feature is the excretion of 16 mg per day of pregnanetriol in Case 1 during the control period and 48 mg per day during ACTH administration. The other patient displayed slightly elevated urinary levels of pregnanetriol with greater than normal response to ACTH. The excretion of tetrahydro S was elevated in Cases 3 and 4 during both periods.

The metabolites of cortisol, tetrahydro E and tetrahydro F were excreted in increased amounts by the patients with Cushing’s syndrome. There was a normal excretion of these metabolites in Case 1. Following ACTH, Cases 2, 3 and 4 demonstrated large increases in the excretion of tetrahydro E and tetrahydro F, consistent with the known hyperreactivity of the adrenal gland in this disease. In Case 1, there was a definite increase in urinary tetrahydro E and tetrahydro F.

**DISCUSSION**

Except for the late onset of the manifestations of the disease, Case 1 presented the classical picture of congenital adrenal hyperplasia (Wilkins 1957). There
was growth acceleration, virilization, an elevated excretion of 17-KS and pregnanetriol. ACTH resulted in a large increase in pregnanetriol excretion, from 16 to 48 mg per day; and cortisone reduced the excretion of pregnanetriol and 17-KS to normal.

The increased excretion of 11-deoxy-KS is in general accord with the observation of Jailer et al. (1955), Masuda (1957), and others. The excretion of androsterone and etiocholanolone were about equal. It has often been noted (Bergstrand et al. 1954; Jailer et al. 1955; Masuda 1957; Jaoudé et al. 1957) that the ratio of androsterone to etiocholanolone may be substantially greater than one. However, in these studies and among other studies (Fukushima & Gallagher 1957; Bush et al. 1957) there were patients with a normal androsterone:etiocholanolone ratio. Thus, the excretion of these metabolites of the C19O2 adrenal androgen(s) is within the accepted pattern of steroid excretion in adrenal hyperplasia.

There has been essential agreement that the excretion of 11β-hydroxyandrosterone is elevated in congenital adrenal hyperplasia. This proved to be so in Case 1. Since this compound is the major metabolite of 11β-hydroxyandrost-4-ene-3,17-dione (Bradlow & Gallagher 1957) and in view of the low excretion of the other 11-oxy-KS, the formation of 11β-hydroxyandrostenedione and by inference androstenedione was increased in Case 1. It is also apparent that 11β-hydroxylation was unimpaired.

The high excretion of pregnanetriol is the hall-mark of congenital adrenal hyperplasia. A marked rise in response to ACTH often occurs (Wilkins et al. 1957) indicating that the capacity of the adrenal gland to respond to ACTH persists. This is substantiated in Case 1 by the increase in pregnanetriol after ACTH and the smaller increase in the excretion of tetrahydro E and tetrahydro F.

The relatively normal excretion of cortisol metabolites does not contradict the hypothesis of impaired 21-hydroxylation since, as Bongiovanni & Eberlein (1958) have pointed out, this normal excretion may be achieved at the expense of increased biosynthetic activity of the adrenal gland. Cope (1959) has presented a patient with congenital adrenal hyperplasia and a normal cortisol secretion rate.

The comparison of the steroid excretion of Case 1 with that of the patients with Cushing’s syndrome does not permit an explanation of the differences in degree of virilization. Cases 3 and 4 who excreted the highest amount of androsterone and etiocholanolone showed little evidence of virilization. Unfortunately Case 2 received ACTH for only one day so that a direct comparison of the amounts of steroids excreted is not valid. However, the uniform increase in the excretion of 11-OHA must indicate that similar pathways of adrenal androgen biosynthesis occurs in Cushing’s syndrome, in Cushing’s syndrome with severe virilization and in virilizing adrenal hyperplasia. Only a know-
ledge of the particular androgen secreted by the adrenal in each instance could explain the differing degree of virilization.

The occurrence of the increased levels of tetrahydro E and tetrahydro F as well as the large increment resulting from ACTH is part of the familiar pattern of Cushing's syndrome. The reversal of the ratio of tetrahydro E:tetrahydro F in Cushing's syndrome and after ACTH has been amply discussed by Gold et al. (1959). The occurrence of tetrahydro S and its increase in response to ACTH has been observed (Dohan et al. 1955) and as with pregnanetriol probably represents only an overflow of their respective precursors during the synthesis of cortisol.

To our knowledge there are only a few cases with post-natal virilizing adrenal hyperplasia in which the steroid excretion has been studied and demonstrated to be the same as in congenital adrenal hyperplasia. Two of the 3 cases described by Brooks et al. (1960) were virilized and all excreted increased amounts of 17-KS and pregnanetriol. There was a major increase in pregnanetriol excretion after ACTH. Greenblatt's (1958) 4 patients had similar biochemical findings.

Dyrenfurth et al. (1958) mentioned one patient, F. L., with post-pubertal adrenal virilism with high 17-KS but pregnanetriol was not determined. It also seems possible that some of the cases of Perloff et al. (1958) with severe hirsutism and marked elevation of the C19O2 and C19O3 fractions might fall into this group.

The pattern of steroid excretion of the patient described by Decourt et al. (1957) and of the patients presented by Jayle et al. (1960) was similar in certain aspects to that of congenital adrenal hyperplasia. There was an increased excretion of pregnanetriol and somewhat increased levels of urinary 17-KS. The excretion of the metabolites of cortisol was low but increased in response to ACTH. However, these patients had only slight hirsutism and no evidence of virilization. Furthermore, as the authors commented, the excretion of the 11-deoxy-17-KS and 11-oxy-17-KS was not similar to congenital adrenal hyperplasia. Thus the relationship of these cases to congenital adrenal hyperplasia rests only upon the demonstration of the increased levels of pregnanetriol and the presumption that this represents the result of relatively decreased 21-hydroxylation.

Congenital adrenal hyperplasia has been classified as one of the hereditary metabolic diseases (Eberlein & Bongiovanni 1960). Since Case 1 demonstrated a steroid excretion pattern consistent with one of the known enzymatic blocks in congenital adrenal hyperplasia, impaired 21-hydroxylation, it is pertinent to inquire if this defect is congenital and whether such a defect could be clinically silent for 6 years. Bergstrand et al. (1960) have shown that the capacity of the adrenal in congenital adrenal hyperplasia to perform 11-hydroxylation decreases with age. Whether a relatively mild defect in 21-
hydroxylation could become more severe with time, thus raising androgen excretion to a level at which clinical manifestations occurred, is of course, only speculative. However, if such events are possible, then the usual occurrence of virilizing adrenal hyperplasia after puberty may be more easily understood, since at puberty the increase in adrenal activity in the presence of defective 21-hydroxylation could result in elevated androgen secretion.

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REFERENCES


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